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Gymnopilus dilepis, a new record in Thailand

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Abstract—Specimens collected in Thailand have been identified as *Gymnopilus dilepis*, based on morphology and ITS molecular analysis. A description and illustration are provided. This is the first record of this fungus from Thailand.

Key words—*Agaricales*, *Cortinariaceae*, gill fungus, taxonomy

Introduction

Karsten (1879) erected the genus *Gymnopilus,* with *Agaricus liquiritiae* Pers. [≡ *G. liquiritiae* (Pers.) P. Karst.] as the type species. *Gymnopilus* has been treated as a member of *Cortinariaceae*, *Agaricales* (Singer 1986). This genus of saprotrophic fungi is distributed worldwide in temperate and tropical regions, usually occurring on dead wood (Singer 1986; Guzmán-Dávalos 2003), and approximately 200 species have been reported (Kirk et al. 2008). Four *Gymnopilus* species—*G. aeruginosus* (Peck) Singer, *G. junonius* (Fr.) P.D. Orton, *G. penetrans* (Fr.) Murrill, and *G. punctifolius* (Peck) Singer—have been reported from Thailand (Chandrasrikul et al. 2011). During a taxonomic survey of macrofungi collected in northern Thailand, we collected specimens that corresponded to the description of *G. dilepis*, a species previously reported from Australia, India, Indonesia, Japan, Malaysia, Papua New Guinea, Sri Lanka, and the United Kingdom (Pegler 1986, Treu 1998, Guzmán-Dávalos

2003, Guzmán-Dávalos et al. 2003, Thomas et al. 2003, Rees et al. 2004, Kasuya et al. 2016). Here we describe and illustrate the morphological characters of the Thai material and provide results from ITS sequence analysis.

Materials & methods

Morphology studies

Basidiocarps were collected from Lampang Province, Thailand, in 2015 and wrapped in aluminum foil or kept in plastic specimen boxes for transport to the laboratory. Notes on macromorphological features and photographs were obtained within 24 h of collection. Color names and codes follow Kornerup & Wanscher (1978). The specimens were dried at 40–45 °C; sections of dried material were mounted in 95% ethanol and rehydrated in distilled water, 3% KOH, or Melzer's reagent for microscopical examination; at least 50 measurements were made of each structure. The collections are deposited in the herbarium of the Research Laboratory for Excellence in Sustainable Development of Biological Resources, Faculty of Science, Chiang Mai University, Thailand (SDBR-CMU).

Molecular studies

Genomic DNA of two specimens was extracted from fresh tissue using a Genomic DNA Extraction Mini Kit (Favorgen, Taiwan). The internal transcribed spacer (ITS) region of the ribosomal RNA gene was amplified by the polymerase chain reaction (PCR) with ITS4 and ITS5 primers under the following thermal conditions: 94 °C for 2 min; 35 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min; and 72 °C for 10 min. PCR products were checked on 1% agarose gels stained with ethidium bromide under UV light and purified using NucleoSpin® Gel and a PCR Clean-up Kit (Macherey-Nagel, Germany), following the manufacturer's protocol. The purified PCR products were directly sequenced. Sanger sequencing was carried out by 1ST Base Company (Kembangan, Malaysia) using the ITS4 and ITS5 primers. Sequences were used to query the GenBank database via BLAST (http://blast.ddbj.nig.ac.jp/top-e.html).

Results

Taxonomic description

Gymnopilus dilepis (Berk. & Broome) Singer, Lilloa 22: 560 (1951). Fig. 1

Pileus 20–50 mm in diameter, convex to plano-convex, surface light orange (6A5) towards the center, pale orange (5A3) to light orange (5A4) towards the margin, squamulose; squamules ruby (12D8) to violet brown (10F8), erect and comparatively denser at the center, sparse and appressed in the middle, sparse or almost absent towards the margin; margin decurved to plane. Context concolorous with the pileus surface. Lamellae adnate to subdecurrent, light orange (5A5) to orange (5A6). Stipe 25–60 × 3–9 mm, central, almost equal or slightly tapering basally, surface pale orange (5A3), becoming dark brown

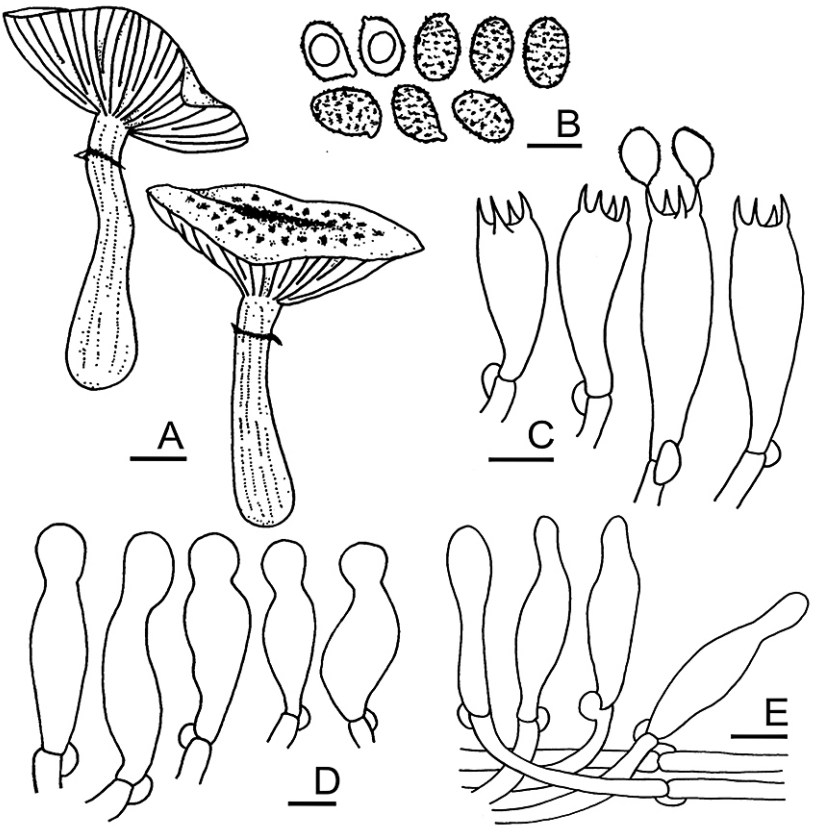

Fig. 1. *Gymnopilus dilepis* (SDBR-CMU-NK0116).
A: basidiocarps; B: basidiospores; C: basidia and basidiospores; D: cheilocystidia; E: caulocystidia.
Scale bars: A = 10 mm, B–D = 5 μm, E = 10 μm.

(6F6) towards base. Veil present. Odor slight. Pileus and stipe surface darkened to brownish orange (6C8) with aqueous KOH. Spore print light brown (6D8).

Basidiospores 6–7.5 × 4.8–6 μm, Q = 1.23–1.5, broadly elliptical to ellipsoid, with obtuse apex, slightly thickened walls, verrucose, warts medium to large, dextrinoid, orange-brown in KOH. Basidia 20–27.5 × 5–8.5 μm, clavate to cylindric-clavate, hyaline, 4-spored, with basal clamp connection, sterigmata ≤5.5 μm long. Pleurocystidia absent. Pseudocystidia 20–27 × 7–8.5 μm, clavate-rostrate or subfusiform, thin-walled, with granulose to homogeneous orange-brown contents. Cheilocystidia 20–30.2 × 8–12 μm, apex 2.2–4.5 μm diam.,

utriform, ranging from clavate with a wide rostrum to lageniform with a short neck and a non-capitate or subcapitate apex, thin-walled, with basal clamp connection, hyaline to pale yellow. Hymenophoral trama subregular, hyphae 2–20 µm diam., thin-walled, hyaline to pale yellow. Subhymenium inflated-ramose. Pileal trama radial, hyphae 2–20.5 µm diam., thin-walled, hyaline to pale yellow. Pileipellis a cutis, hyphae 3–15 µm diam., thin-walled, coarsely encrusted with brown pigment. Stipe trama composed of hyphae 2–25 µm diam., parallel, thin-walled, pale yellow. Stipitipellis a cutis, hyphae 2–12 µm diam., thin-walled with pale yellow to brown wall pigment. Caulocystidia 18.2–65 × 5.5–14.5 µm, cylindrical, clavate, narrowly utriform, with obtuse or subcapitate apex, hyaline, some with granulose, pale yellow or orange-brown content, thin-walled, in tufts at the stipe apex. Clamp connections present on all hyphae.

Specimens examined—**THAILAND**, **Lampang Province**, Mae Moh District, 18°40′52″N 98°52′10″E, elevation 550 m, on dead wood in a deciduous forest, 8 November 2015, Suwannarach N & Lumyong S (SDBR-CMU-NK0116; GenBank KX639496); Kumla J & Suwannarach N (SDBR-CMU-JK0142; GenBank KX639497).

Molecular analysis

The ITS sequences of specimens SDBR-CMU-NK0116 (644 bp) and SDBR-CMU-JK0142 (638 bp) were deposited in GenBank. All ITS sequences obtained in this study showed 100% similarity with *G. dilepis* KT368680 and KT368682, from Japan.

Discussion

Both specimens collected in northern Thailand were initially identified as *G. dilepis* based on descriptions by Guzmán-Dávalos (2003) and Thomas et al. (2003). This species is easily distinguished from *G. penetrans*, which produces a gray-brown to dark brown pileus with a white to grayish-white tomentose-arachnoid velum covering the whole surface (Holec 2005). The smaller basidiospores (4.0–6.0 × 3.5–5.0 µm) of *G. punctifolius* and the longer basidiospores (8.0–10.5 × 5.5–7.2 µm) of *G. junonius* clearly distinguish these two species from *G. dilepis* (Singer 1951; Holec 2005).

Gymnopilus dilepis is closely related to *G. purpuratus* (Cooke & Massee) Singer and *G. norfolkensis* B.J. Rees & Lepp, from which it is distinguished by differences in spore size: basidiospores of *G. purpuratus* differs are longer (7.5–8.7 × 4.8–5.7 µm; Rees et al. 2004) while those of *G. norfolkensis* are narrower (6.4–7.2 × 4.0–5.2 µm; Rees and Lepp 2000). *Gymnopilus dilepis* also resembles *G. lepidotus* Hesler, but the two species differ in the form of

the cheilocystidia; additionally *G. lepidotus* is known only from Mexico and the United States (Hesler 1969, Guzmán-Dávalos 2003, Guzmán-Dávalos et al. 2003).

Our molecular analysis also confirmed the two Thai specimens as representing *G. dilepis*. The combination of morphological and molecular characters confirms *G. dilepis* as a new record for Thailand.

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